REVIEW

Structure and function of the SPRY/B30.2 domain proteins involved in innate immunity

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Abstract: The SPRY domain is a protein interaction module found in 77 murine and ~100 human proteins, and is implicated in important biological pathways, including those that regulate innate and adaptive immunity. The current definition of the SPRY domain is based on a sequence repeat discovered in the splA kinase and ryanodine receptors. The greater SPRY family is divided into the B30.2 (which contains a PRY extension at the N-terminus) and "SPRY-only" sub-families. In this brief review, we examine the current structural and biochemical literature on SPRY/B30.2 domain involvement in key immune processes and highlight a PRY-like 60 amino acid region in the N-terminus of "SPRY-only" proteins. Phylogenetic, structural, and functional analyses suggest that this N-terminal region is related to the PRY region of B30.2 and should be characterized as part of an extended SPRY domain. Greater understanding of the functional importance of the N-terminal region in "SPRY only" proteins will enhance our ability to interrogate SPRY interactions with their respective binding partners.

Keywords: SPRY domain; PRY; B30.2 domain; TRIM; BTN; SPSB; SOCS box; IgG; innate immunity; structure/function

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Abbreviations: Ash2L, ash2 (absent, small, or homeotic)-like; BTN, butyrophilin; DDX1, DEAD (Asp-Glu-Ala-Asp) box helicase 1; hnRNP, heterogeneous nuclear ribonucleoprotein; iNOS, inducible nitric oxide synthase; MID1, midline 1; RIG-I, retinoic acid-inducible gene I; HIV, human immunodeficiency virus; MEFV, Mediterranean fever; MLV, murine leukemia virus; NMR, nuclear magnetic resonance; IRF, interferon regulatory factor; NFκB, nuclear factor of kappa light polypeptide gene enhancer in B-cells; NO, nitric oxide; FMF, familial Mediterranean fever; Fbxo45, F-box protein 45; IgG, immunoglobulin G; PYD, pyrin domain; RNF135, ring finger protein 135; Rbx2, RING box protein 2; RyR, ryanodine receptor; RING, really interesting new gene; RBCC, RING/B-box/coiled coil; SPRY, in splA kinase and ryanodine receptor; SOCS, suppressor of cytokine signaling; SPSB, SPRY domain-containing SOCS box; TRIM, tripartite motif; TLRs, toll-like receptors.

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Introduction

Approximately 100 human and 77 murine proteins with a diverse range of functions contain a SPRY/B30.2 protein-interaction domain and these can be divided into 11 subfamilies on the basis of amino acid sequence similarity or the presence of additional protein domains.[1] The B30.2 domain (also known as RFP-like or PRYSPRY) was originally identified based on sequence homology to a protein encoded by the B30.2 exon located within the major histocompatibility complex (MHC) Class I region[2] and was later defined by the presence of three highly conserved sequence motifs (LDP, WEVE, and LDYE).[3] Concurrently the SPRY domain was identified based on a sequence repeat in the dual specificity kinase spore lysis A found in *Dictyostelium discoidueum* and in all three mammalian Ca^{2+}-release channel ryanodine receptors (RyR).[4] A comparison of the two domains by Rhodes and colleagues revealed that the second two motifs were conserved across B30.2 and SPRY domains, leading to the conclusion that B30.2 domains consisted of a SPRY domain preceded by an N-terminal region containing a "PRY" motif.[1]

There are now more than 1600 eukaryotic proteins containing the SPRY domain annotated in the SMART database. 516 of these are B30.2 domains whilst the remaining members of the family, which are not preceded by any recognizable PRY motif at the N-terminus, are denoted as "SPRY-only" domains, and are evolutionarily more ancient than their B30.2 counterparts.[1,5–7] However, recent structural and biochemical studies have suggested that SPRY-only domains are preceded by a subdomain that is structurally similar to the PRY region. The potential importance of this region has been noted previously[8,9] and here we discuss its role in the structure, function, and evolution of the greater SPRY family.

All SPRY/B30.2 structures have a bent β-sandwich fold comprised of two β-sheets. The first crystal structure of a human B30.2 domain (residues 1–201) showed that the PRY domain consists of three β-strands (β1–β3) tightly packed against ten other β-strands (β4–β13), which form the SPRY component of the B30.2 domain. The β-strands are linked by loops of varying lengths and form two anti-parallel β-sheets of six and seven strands, respectively, arranged in a bent β-sandwich fold.[10] Simultaneously, a solution structure of the murine "SPRY-only" protein SPRY domain-containing SOCS box (SPSB) 2 and a crystal structure of GUSTAVUS (the *Drosophila* homologue of SPSB1 and 4) revealed a similar structure to the B30.2 domain, with three β-strands from the N-terminal region packing against the β-strands of the SPRY domain, as well as a number of loop regions that were demonstrated to be important for binding interactions.[8,11]

The SPRY/B30.2 domains mediate protein–protein interactions, although in the majority of cases the interacting partners remain unknown, as do the molecular determinants of binding specificity. Nonetheless, it is becoming increasingly clear that SPRY/B30.2 proteins are involved in many important signaling pathways. For example, amongst "SPRY-only" proteins, the DEAD box protein DDX1 and heterogeneous nuclear ribonucleoprotein (hnRNP) proteins are involved in RNA processing, while Ash2L is involved in the regulation of histone H3 lysine 4 (K4) methylation.[12–15] The second of three SPRY domains in RyR1 makes an intramolecular interaction with the alternatively spliced residues and neighboring basic residues of RyR1 to regulate excitation coupling in skeletal muscle.[16] The B30.2 domain-containing protein Tripartite motif (TRIM) 7 is involved in glycogen biosynthesis[17,18] and TRIM10 is essential for red blood cell membrane integrity and terminal erythroid cell differentiation.[19] The Rfpl4 (ret finger protein-like 4) B30.2 domain interacts with cyclin B, and is important for oocyte and early embryonic development,[20,21] while TRIM18 (MID1) is thought to associate with cytoplasmic microtubules, with mutations in human TRIM18 linked to X-linked Optiz syndrome (manifested as cleft lip, heart defects, and other midline abnormalities).[22–24] In addition, many SPRY/B30.2 proteins (members of the TRIM, BTN, and SPSB families) appear to be involved in innate immunity, although only a few of these have been well characterized.

SPRY/B30.2 domain-containing proteins involved in innate cellular responses

Leukocytes of the innate immune system such as mast cells, eosinophils, basophils, and natural killer cells, as well as the phagocytic macrophages, neutrophils, and dendritic cells, are responsible for identifying and eliminating pathogens.[25] They are activated by pathogen-associated molecular patterns (PAMPs) in invading microbes, which bind to pattern recognition receptors (PRRs) such as the transmembrane Toll-like receptors (TLRs), and the cytoplasmic retinoic acid-inducible gene-I (RIG-I) and NOD-like receptors (RLRs and NLRs).[26–29] Recognition of PAMPs by TLRs and RLRs activates signaling pathways to produce type 1 interferons and other pro-inflammatory cytokines.[26,28] Increasingly, TRIM proteins such as pyrin, TRIM21, TRIM25, TRIM27, TRIM30α, and Riplet are being found to play key roles in regulating these signaling cascades.[30–38]

Members of the TRIM protein family are characterized by a RING/B box/coiled-coil (RBCC or tripartite motif) core, with approximately half containing a B30.2 domain at the C-terminus.[39] The RING (Really Interesting New Gene) domain confers E3 ligase activity by recruitment of the E2-ubiquitin conjugate, while the B30.2 domain is responsible for binding the substrate molecule. Ubiquitin is then transferred to the substrate to initiate the process of

polyubiquitination.[40] The coiled-coil domain promotes the homo-oligomerization of TRIM7, TRIM5α, and TRIM25[17,41–43] and is thus likely to function similarly across the TRIM family proteins. E3 ubiquitin ligase activity is often coupled with SPRY/B30.2 domains, which are responsible for targeting substrate proteins for polyubiquitination, resulting in inhibition or activation of signaling.

TRIM25 positively regulates the antiviral response by activating RIG-I in the initial stages of viral infection, and promoting degradation of mitochondrial antiviral signaling (MAVS) protein in the latter signal transduction phase.[35] The B30.2 domain is necessary and sufficient for the interaction with RIG-I,[34,44] but its role in the MAVS interaction remains unknown. Riplet/RNF135 is a TRIM-like protein that also activates RIG-I.[38,45] Activation of the RIG-I signaling cascade ultimately results in an antiviral response, characterized by the production of type I interferons. Recently, the TRIM27 (also known as Ret finger protein (Rfp)) B30.2 domain was shown to bind the nucleotide-binding domain of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), and recruit it for K48-linked ubiquitination and subsequent proteasomal degradation.[46] TRIM27 is also thought to negatively regulate class II phosphotidylinositol-3-kinase C2β (PI3KC2β) signaling in mast cells and $CD4^+$ T cells[47,48] and inhibit the inhibitor of NF-κB (IκB) kinase family members via ubiquitin-independent mechanisms in response to virus- and cytokine-induced inflammatory signaling.[36] TRIM30α negatively regulates TLR-mediated NF-κB activation by promoting TAK1-binding protein (TAB) 2 and TAB3 degradation,[37] and is also thought to negatively regulate pro-interleukin-1β processing by attenuating production of reactive oxygen species, and thereby negatively regulating the NLR family pyrin domain-containing 3 (NLRP3) inflammasome.[49]

The following section highlights the biological roles of key SPRY/B30.2 domains for which we have structural information and which are involved in regulating the innate immune response (Fig. 1).

In Old World Monkeys, the B30.2 domain of TRIM5α binds the capsid of the human immunodeficiency virus-1 (HIV-1) and murine leukemia virus (MLV), conferring immunity. Rhesus TRIM5α prevents reverse transcription of the viral genome and its subsequent transport to the nucleus by causing the premature uncoating of the viral capsid.[50,51] Although exactly how this process inhibits viral replication remains unknown, inhibition of the proteasome alters the localization of TRIM5α from the cytoplasm to nuclear bodies and restores HIV-1 reverse transcription, suggesting that degradation of the capsid, viral RNA or other important viral enzymes by the proteasome may be responsible.[52] In humans, however, TRIM5α is unable to restrict HIV-1 replication, apparently due to a very weak interaction between TRIM5α and the HIV-1 capsid,[51] although it can inhibit strains of MLV.[53,54] A single amino acid substitution in the PRY region of human TRIM5α (Arg332 to Pro; found in rhesus macaque TRIM5α) can confer the ability to restrict HIV-1, with variable loop regions from the SPRY region (356–491) also involved in binding.[55–57] Modeling species-specific amino acid differences onto the recently published hybrid NMR/crystal structure of the rhesus TRIM5α B30.2 domain suggests that both the PRY and SPRY regions are involved in binding to capsid. The extended and hyper-variable loop region of the TRIM5α B30.2 domain, which the authors denote as v1, is unique when compared to other B30.2 domain structures. The authors confirmed by NMR that v1 and other loop regions of the TRIM5α B30.2 domain are required for interaction with the target capsid proteins and suggested that binding occurs in a similar manner to the recognition of antigen by immunoglobulin (Ig)M antibodies.[58] Therefore, both the PRY and SPRY regions of the TRIM5α B30.2 domain determine the specificity of retroviral restriction.

Pyrin/TRIM20/Marenostrin is encoded by the MEFV gene and is an important regulator of innate immunity. Pyrin is an interferon-inducible protein and regulates pro-interleukin-1β processing and secretion.[59] Instead of the RING domain typical of most TRIM proteins, pyrin contains a PYD domain (pyrin domain) at the N-terminus, which facilitates homo and heterodimer formation with other death domains, such as the caspase activation and recruitment domain (CARD)s.[60] Mutations that cluster within the PRY and SPRY encoding regions in humans are associated with familial Mediterranean fever (FMF).[10,61] Affinity purification and coimmunoprecipitation experiments have demonstrated an interaction between the B30.2 domain of pyrin and the protease domain of capase-1.[30,61] However, whether this interaction leads to the activation or inhibition of caspase-1 (i.e., whether missense mutations observed in FMF are activating or inactivating) remains to be clarified, with examples of both outcomes reported in the literature.[61–63]

TRIM21/Ro52/SS-A1 is upregulated by type I and II interferon and is a potent self-antigen recognized by autoantibodies. Although the autoantibodies are found primarily in the sera of patients with Systemic Lupus Erythematosus (SLE) and Sjögren's Syndrome (SS), they are also found in patients with other systemic autoimmune diseases such as rheumatoid arthritis and systemic sclerosis.[64–68] While the role of TRIM21 in the etiology of SLE, SS, and other inflammatory diseases remains unknown, it has been suggested that autoantibodies enter the cell during viral infection and may inhibit TRIM21 E3 ligase activity leading to dysregulation of type I interferon production.[69,70]

The TRIM21 B30.2 domain has been shown to interact with interferon regulatory factors (IRF) 3 and 7 in a phosphorylation-dependent manner, targeting them for polyubiquitination and proteasomal

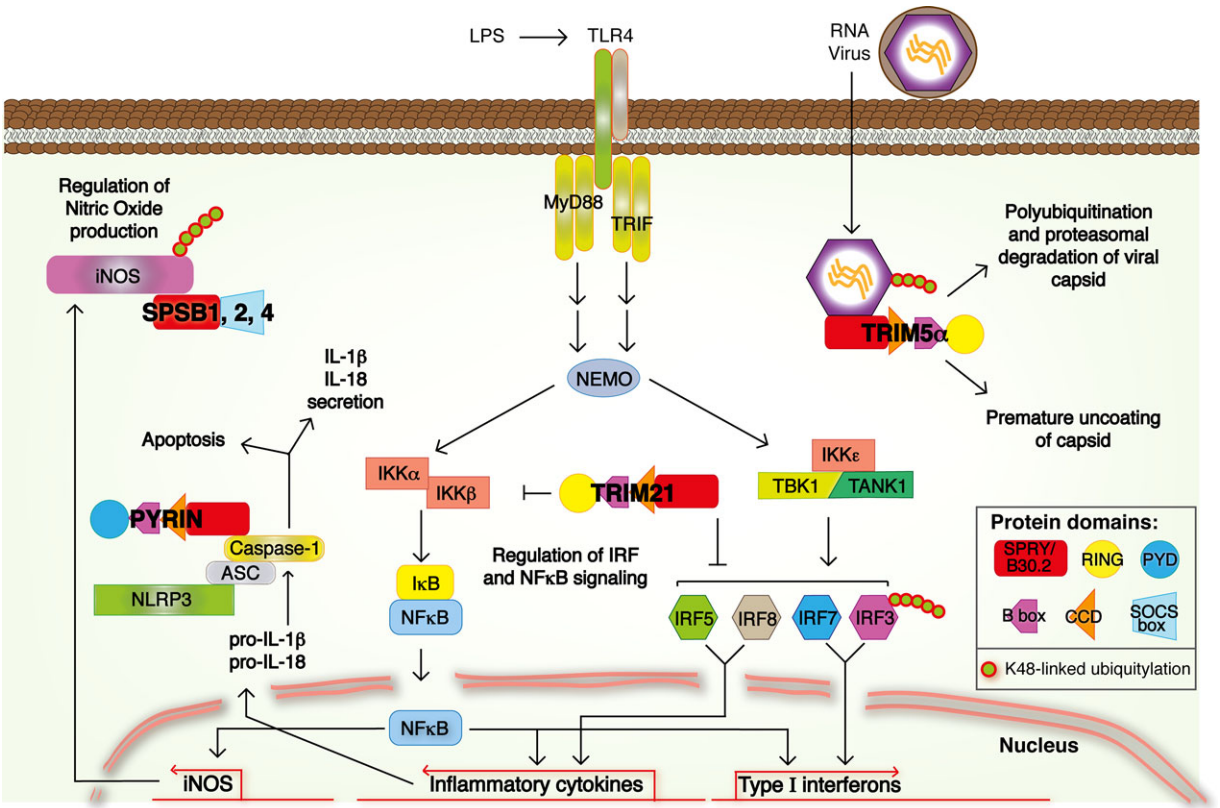

Figure 1. The role of SPRY/B30.2 domain-containing proteins in innate immune responses. Activation of Toll-like receptor (TLR) 4 signaling by pathogen-associated molecular patterns (PAMP) such as lipopolysaccharide (LPS) occurs via MyD88 (myeloid differentiation primary response protein)–dependent and independent pathways, the latter via the TRIF (TIR domain-containing adapter molecule 1) complex. Both pathways activate NEMO (NFκB essential modulator, also known as IKKγ), which in turn activates the IKK complex (IκB kinases, α and β), which inhibits IκB (inhibitor of NF-κB), releasing NFκB (nuclear factor-κB) for translocation into the nucleus. The NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome activates caspase-1 in an ASC (apoptosis-associated speck-like protein containing a CARD)-dependent manner, which then processes pro-IL-1β and pro-IL-18 into their active forms. The B30.2 domain of pyrin interacts with caspase-1 to regulate the cleavage of pro-IL-1β and pro-IL-18. NEMO also activates the IKKε, TANK1 (TRAF family member-associated NF-kappa-B activator 1), TBK1 (TANK-binding kinase 1) complex, leading to phosphorylation- and dimerization-mediated activation of the interferon regulatory factors (IRFs) and transcription of target genes. RING, Really Interesting New Gene; PYD, pyrin domain; CCD, coiled-coil domain; SOCS box, suppressor of cytokine signaling.

degradation, thereby limiting the production of type I interferon following viral infection.[33,71,72] More recently, TRIM21 has been suggested to play a very different role in the early antiviral response. It is thought that TRIM21 binds to cytosolic antibody-virus complexes and targets them for proteasomal degradation.[73] The TRIM21 B30.2 domain interacts directly with the F_c region of IgG of multiple species, exhibiting high affinity (K_D37 nM) interactions with human IgG_1, IgG_2, and IgG_4. The B30.2 domains of murine and human TRIM21 in complex with the F_c region of IgG (PDB[74] id 2VOL and 2IWG, respectively) demonstrate that residues from the PRY and SPRY regions form hydrogen bonds with residues from the F_c region that are distinct from those involved in binding to other F_c receptors.[31,32,75] Unlike the SPRY domain-containing SOCS box (SPSB)s 1, 2, and 4, which recognize a linear NNN-containing motif (discussed below),[76] the target residues on the F_c molecule are not linear but form a conformational epitope. Crucially, comparison of the unbound and bound structures of the TRIM21 B30.2 domain revealed that the binding site does not adhere to an induced fit model but rather has a pre-formed binding pocket.[75] FMF-causing mutations within the human pyrin PRY (and SPRY) regions,[10,61] as well as the defining role of PRY residues in TRIM5α HIV restriction,[55] further add to growing evidence that the PRY region contributes key residues in interactions with target proteins.

SPRY domain-containing SOCS box proteins (SPSBs)

Of the "SPRY-only" proteins, the SPSB family has also emerged as having a role in regulating the innate effector response. Similarly to many TRIM proteins, the SPSBs act as adaptor proteins to facilitate substrate ubiquitination by E3 ubiquitin ligases. In

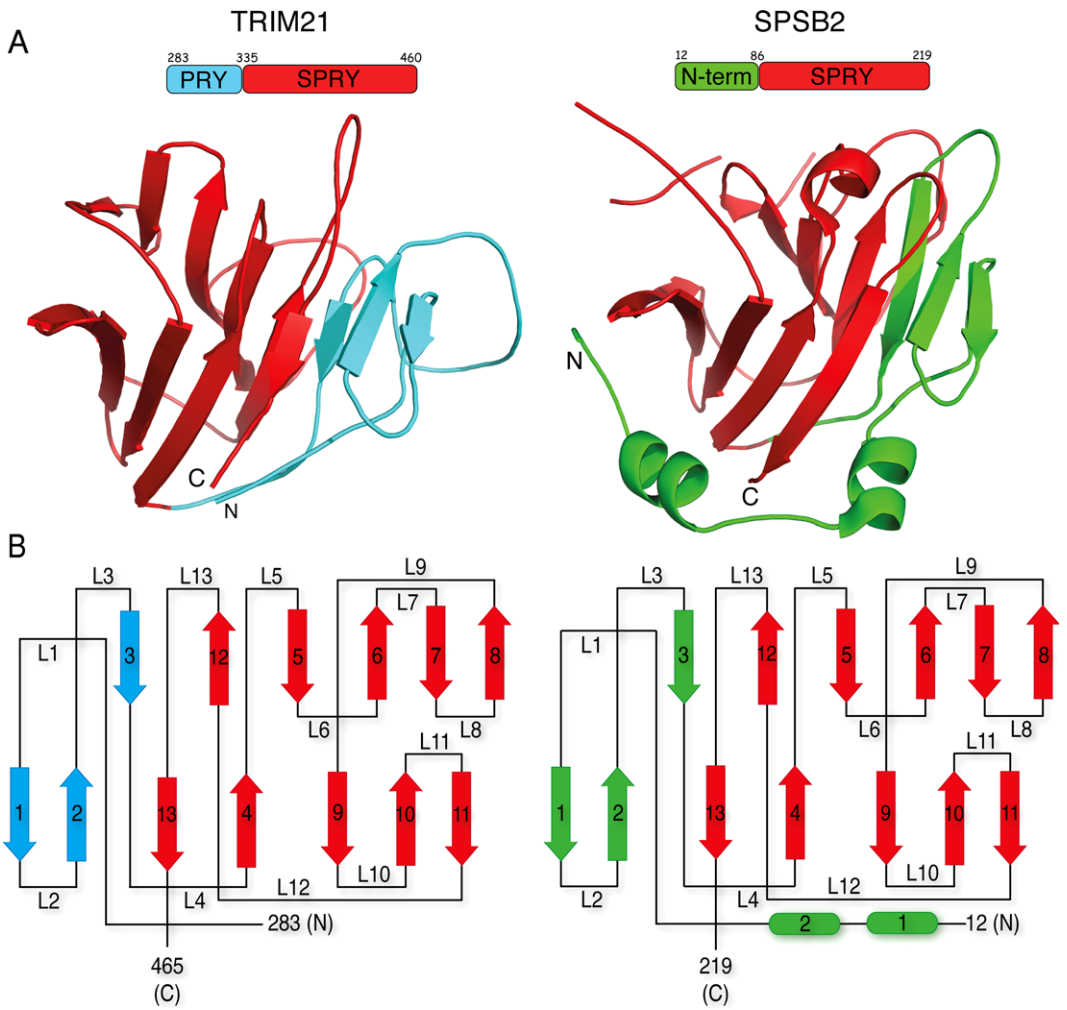

Figure 2. Structural conservation of the PRY region of TRIM21 and the N-terminal region of SPSB2 suggests these regions are evolutionarily and functionally related. A: Cartoon representation of the TRIM21 B30.2 domain$_{283–465}$ (PDB: 2VOK) and SPSB2 SPRY domain$_{12–219}$ (PDB: 3EK9) crystal structures. The SPRY regions of both proteins are in red, while the PRY and N-terminal regions are in cyan and green, respectively. The amino acid numbers and domain architectures are shown. B: Topology diagrams of the TRIM21 B30.2 (left) and SPSB2 SPRY (right) domains with the structural elements colored as in (A). α-helices and β-strands are numbered, and shown as rounded rectangles and arrows, respectively. Loop regions are numbered L1–L13. Based on comparisons with all SPRY structures to date, we consider the first beta strand of SPSB2 as too distorted to be a true secondary element, therefore the N-terminal region is shown here as containing 3 β-strands.

mammals, the family consists of four members, SPSB 1, 2, 3, and 4, which are characterized by a C-terminal SOCS box, a central SPRY domain and a variable N-terminal region. The SPRY domains of SPSB 1, 2, and 4 interact with a DINNN peptide motif in the N-terminus of inducible nitric oxide synthase (iNOS), while the SOCS box recruits elongin B and elongin C, and, together with the adaptor protein cullin 5 and Rbx2, forms an E3 ubiquitin ligase complex. The SPRY domain is therefore responsible for binding iNOS,[77,78] targeting it for SOCS box-mediated polyubiquitination and subsequent proteasomal degradation.[79–81] iNOS is a key effector of the innate immune response, producing nitric oxide (NO) in response to infection, which, along with other reactive nitrogen species, is toxic to invading microbes.[82] Fsn, the *Drosophila* homologue of Fbxo45, also binds the DINNN motif,[83] but has not yet been shown to regulate iNOS in mammalian cells.

The N-terminal region of SPSB2 contains two α-helices and three β-strands, which, together with the eleven β-strands of the SPRY region, form a single modular entity.[9] Consistent with this, Arg68 in the SPSB2-SPRY N-terminal region forms a hydrogen bond with the third Asn of the DINNN peptide within iNOS/VASA.[79,84] Arg68 is conserved across the Fbxo45, SPSB1, SPSB2, and SPSB4 SPRY domains, all of which have been shown to interact with the DINNN peptide.[76,83,84] On this basis, we predict that deletion of the N-terminal 85 amino acid residues would significantly disrupt the SPRY domain structure. Structural alignments of the N-terminal region of "SPRY-only" proteins and the PRY region of B30.2 domains show conservation of secondary structural

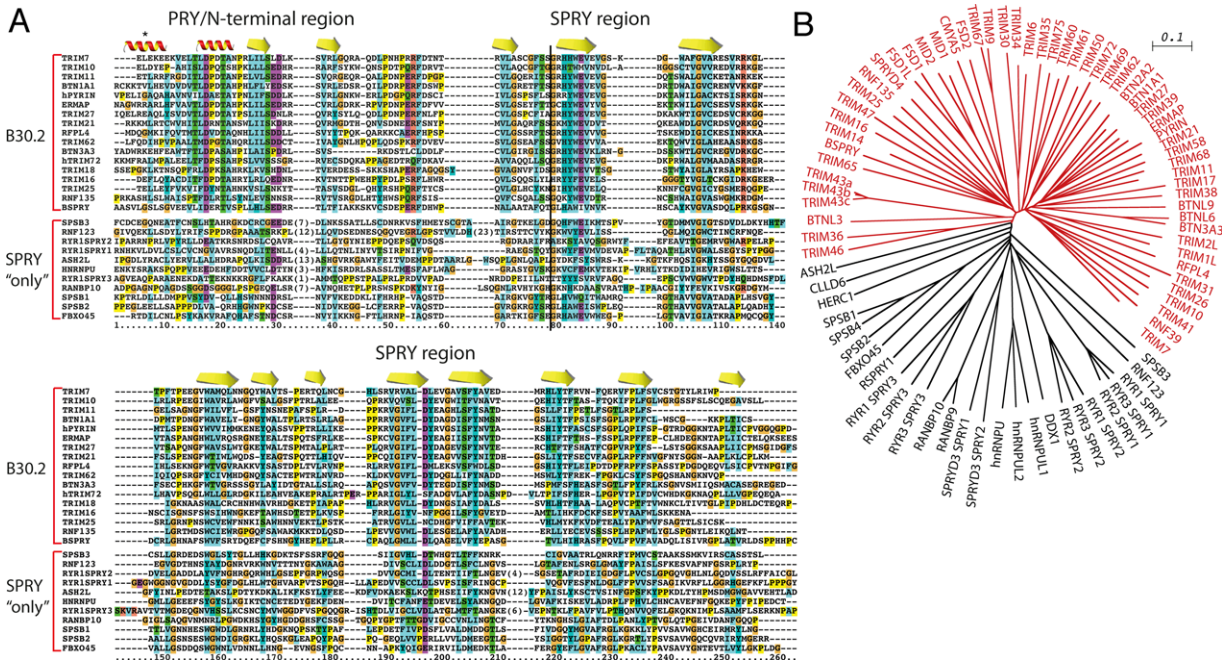

Figure 3. A: Sequence alignment of select members of the murine B30.2 and "SPRY-only" domain family. Sequences were selected from across the SPRY/B30.2 family, sourced from Genbank and aligned using ClustalX 2.0.12. Secondary structural elements positioned above the alignment correspond to residues from the TRIM21 crystal structure.[75] The BLACK line denotes the beginning of the SPRY region. An extra α-helix (denoted by *) was resolved at the N-terminus of human TRIM72 and murine SPSB2.[9,86] B: The B30.2 sub-family clusters together and shares a common ancestral gene. Phylogenetic tree constructed from B30.2 (PRY and SPRY) and "SPRY-only" (N-terminal and SPRY) sequences sourced from Genbank and aligned using ClustalX 2.0.12.[88] An unrooted phylogenetic tree was then generated using Quicktree v1.1.[89] The scale refers to the number of millions of years since the sequences last shared a common ancestor. The RYR family members each contain three SPRY domains, which are distinguished by their relative location within the protein domain architecture, that is, "SPRY1" denotes the most N-terminal of the SPRY domains, with "SPRY2" in the middle and "SPRY3" nearest to the C-terminus. SPRYD3 proteins contain two SPRY domains and are labeled as SPRY1 and SPRY2, respectively. All sequences are from mouse, with the exception of pyrin and TRIM72, for which the human sequence was used.

elements (data not shown). Together, the existing data indicate that the N-terminus is an integral part of the SPSB SPRY domain, just as the PRY region is, in the B30.2 domain of TRIM21 and other B30.2 domain-containing proteins.

Structural comparisons of PRY and N-terminal regions

Examination of the crystal structures of the B30.2 domains of gene 19q13.4.1 (PDB: 2FBE), human TRIM72 (PDB: 3KB5), human pyrin (PDB: 2WL1), and murine and human TRIM21 (PDB: 2VOK and 2IWG, respectively)[9,10,15,75,76,85,86] reveals that the PRY and SPRY regions together form a single modular domain. Structures of the "SPRY-only" proteins Ash2L, and SPSBs 1, 2, and 4, have also been solved. In each of these structures, it can be seen that there is a region of ~60 amino acid residues immediately preceding the SPRY sequence that is structurally similar to the PRY subdomain.[8,9,15,76] As in the PRY domain, these N-terminal domains are all composed of four β-strands and exhibit homologous connectivity. Figure 2 demonstrates the structural similarity between the SPSB2 N-terminal region (green) and the TRIM21 PRY region (cyan). Structural alignments performed by overlaying the TRIM21 B30.2 domain and the SPSB2 N-terminal region and SPRY domain structures using the pairwise alignment function on the DALIweb server[87] yielded an overall RMSD (root-mean-square deviation) of 2.8 Å ($mSPSB2_{12\text{-}219}$ and $mTRIM21_{283\text{-}465}$). This level of structural similarity was consistent across all "SPRY-only" and B30.2 structures deposited in the PDB (data not shown). The position of loop 1 relative to β-strand 3 is likely to be conserved across the SPRY/B30.2 family, as all known structures show similar backbone to backbone hydrogen-bonding between the two regions. This suggests that the 60 residues N-terminal to the SPRY motif in "SPRY-only" proteins may be functionally and evolutionarily related to the PRY region of B30.2 domains.

Prior to any structural information, the lack of sequence homology between the N-terminal SPRY region and PRY [Fig. 3(A)] led to the conclusion that the PRY is a separate domain that was added to the SPRY domain, to form the B30.2 exon concurrently with the evolution of the adaptive immune system.[1]

In fact, multiple sequence alignments of all murine N-terminal sequences show conservation of sequence only between related family members and no obvious overall similarity to each other or to the PRY domain. However, the available structural data show that the PRY and N-terminal regions (in B30.2 and SPRY-only proteins, respectively) are structurally homologous despite the lack of sequence similarity.

The SPRY domain is always preceded by either a PRY or N-terminal segment and structurally these segments appear to form one contiguous unit with the SPRY domain. Therefore, we suggest that it is the PRY/SPRY or N-terminal/SPRY pairs that form the true functional "domain." Where such data exist, structure/function studies also indicate that PRY/SPRY or N-terminal/SPRY pairs form the minimal functional unit; for example the iNOS binding site on SPSB2 consists of residues from both the N-terminal and SPRY regions, and deletion analyses have shown that neither the N-terminus (residues 1–85) nor the SPRY domain (residues 86–219) alone was able to co-immunoprecipitate iNOS.[80] Similarly, the PRY and SPRY domains are both required for TRIM21 to bind to the F_c region of IgG.[31]

Insights from phylogenetic analysis

The B30.2 domain to date, has only been found in vertebrates with an adaptive immune system, while the "SPRY-only" domain is evolutionarily ancient and can be found in animals, plants and fungi.[1] To investigate the phylogenetic relationships among SPRY, N-terminal, PRY, and B30.2 regions, sequences were obtained from the NCBI database and aligned using the multiple sequence alignment program ClustalX 2.0.12[88] [Fig. 3(A)]. Further analyses utilized the tree construction program Quicktree v1.1[89] [Fig. 3(B)]. Analysis of all murine SPRY and B30.2 domains confirms that the SPRY sequence repeat is highly conserved between the "SPRY-only" and B30.2 domains [Fig. 3(A)]. Additionally, the PRY regions of all B30.2 domains display a high level of sequence conservation [Fig. 3(A)], suggesting that the current complement of B30.2 domains all derived from the genetic expansion of a single ancestral B30.2 domain exon. This is clearly visible in the phylogenetic tree of the murine SPRY/B30.2 domain family shown in Figure 3(B) and is consistent with previous phylogenetic analyses of the B30.2 family.[1]

The functional importance of the N-terminal region preceding "SPRY-only" domains and the relationship with the PRY region of B30.2 domains suggest that the ancestral PRY region was simply one of numerous, variable N-terminal regions of ancient SPRY domains. Presumably its association with the TRIM and BTN families and the subsequent genetic expansion of these families has led to a profusion of the PRY-containing B30.2 domain, in comparison to its N-terminal cousins.

Conclusions

The current distinction between the SPRY and B30.2 domains appears not to be supported by existing structural and functional data. The importance of the N-terminus of SPSB2 for binding iNOS, and the PRY region of TRIM21 for binding the F_c region of IgG, as well as the structural conservation between the two, suggests that these components are evolutionarily and functionally related. Structural alignments between the N-terminal regions of other SPSB structures and the PRY regions of pyrin, TRIM72, and gene 19q13.4.1 confirm that similar levels of structural conservation exist between the B30.2 and "SPRY-only" families (data not shown).

B30.2 domain and "SPRY-only" proteins are increasingly being found to have important biological roles, and efforts to identify interacting partners and understand the downstream consequences of those interactions are likely to grow significantly. Understanding the true domain boundaries and how they can be manipulated has important consequences for many of these experiments.

The original definition of the SPRY/B30.2 domains was based on sequence analyses.[2–4] Since then, the publication of a number of SPRY/B30.2 domain structures, both alone and in complex with an interacting partner, indicates that this definition requires revision. We suggest that an N-terminal extension of 60 amino acids be considered as part of the SPRY domain to include all of the sequence that clearly forms a single functional module, and redefine the SPRY domain as "a recognizable and conserved sequence repeat preceded by a more variable N-terminal region of approximately sixty amino acids". On this basis, B30.2 proteins should be considered as a SPRY subfamily with the PRY region as one variant of the N-terminal region, not a separate domain added in vertebrates.[9] We believe that updating the definition of the SPRY domain, in particular clarifying the domain boundaries, will lead to greater success in biochemical and structural studies in the field.

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